



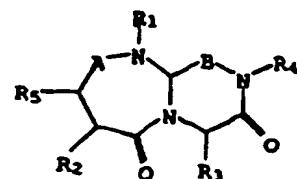
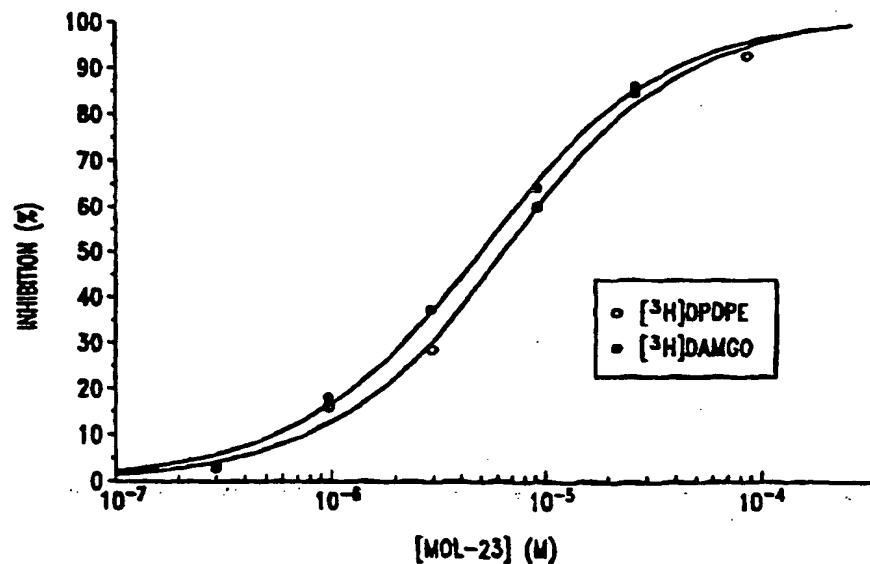
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07D 487/04, A61K 31/495 // (C07D 487/04, 241:00, 239:00)		A1	(11) International Publication Number: WO 97/15577 (43) International Publication Date: 1 May 1997 (01.05.97)
(21) International Application Number: PCT/US96/17053 (22) International Filing Date: 24 October 1996 (24.10.96)		(81) Designated States: AU, CA, JP, KR, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(30) Priority Data: 08/549,007 27 October 1995 (27.10.95) US		Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(71) Applicant: MOLECUMETICS LTD. [US/US]; Suite 400, 2023 - 120th Avenue N.E., Bellevue, WA 98005 (US).			
(72) Inventor: KAHN, Michael; 10916 - 80th Place N.E., Kirkland, WA 98034 (US).			
(74) Agents: HERMANN, Kari, R. et al.; Seed and Berry LLP., 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).			

(54) Title: REVERSE-TURN MIMETICS AND METHODS RELATING THERETO

(57) Abstract

Conformationally constrained compounds of formula (I) which mimic the secondary structure of reverse-turn regions of biologically active peptides and proteins are disclosed. Such reverse-turn mimetics have utility over a wide range of fields, including use as diagnostic and therapeutic agents. Libraries containing the reverse-turn mimetics of this invention are also disclosed, as well as methods for screening the same to identify biologically active members.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

REVERSE-TURN MIMETICS AND METHODS RELATING THERETO

5 Technical Field

The present invention relates generally to reverse-turn mimetics and to a chemical library of reverse-turn mimetics.

10 Background of the Invention

Random screening of molecules for possible activity as therapeutic agents has occurred for many years and resulted in a number of important drug discoveries. While advances in molecular biology and computational chemistry have led to increased interest in what has been termed "rational drug design," such techniques have not proven as fast or reliable as initially predicted. Thus, in recent years there has been a renewed interest and return to random drug screening. To this end, particular strides having been made in new technologies based on the development of combinatorial chemistry libraries, and the screening of such libraries in search for biologically active members.

In general, combinatorial chemistry libraries 25 are simply a collection of molecules. Such libraries vary by the chemical species within the library, as well as the methods employed to both generate the library members and identify which members interact with biological targets of interest. While this field is still young, methods for 30 generating and screening libraries have already become quite diverse and sophisticated. For example, a recent review of various combinatorial chemical libraries has identified a number of such techniques, including the use of both tagged and untagged library members (Janda, *Proc. Natl. Acad. Sci. USA* 91:10779-10785, 1994).

To date, combinatorial chemistry libraries have generally been limited to members of peptide or nucleotide origin. To this end, the techniques of Houghten et al. illustrate an example of what is termed a "dual-defined 5 iterative" method to assemble soluble combinatorial peptide libraries via split synthesis techniques (*Nature (London)* **354**:84-86, 1991; *Biotechniques* **13**:412-421, 1992; *Bioorg. Med. Chem. Lett.* **3**:405-412, 1993). By this technique, soluble peptide libraries containing tens of 10 millions of members have been obtained. Such libraries have been shown to be effective in the identification of opioid peptides, such as methionine- and leucine-enkephalin (Dooley and Houghten, *Life Sci.* **52**, 1509-1517, 1993), and a N-acylated peptide library has been used to 15 identify acetalins, which are potent opioid antagonists (Dooley et al., *Proc. Natl. Acad. Sci. USA* **90**:10811-10815, 1993). More recently, an all D-amino acid opioid peptide library has been constructed and screened for analgesic activity against the mu ("μ") opioid receptor (Dooley 20 et al., *Science* **266**:2019-2022, 1994).

While combinatorial libraries containing members of peptide and nucleotide origin are of significant value, there is still a need in the art for libraries containing members of different origin. For example, traditional 25 peptide libraries to a large extent merely vary the amino acid sequence to generate library members. While it is well recognized that the secondary structures of peptides are important to biological activity, such peptide libraries do not impart a constrained secondary structure 30 to its library members.

To this end, some researchers have cyclized peptides with disulfide bridges in an attempt to provide a more constrained secondary structure (Tumelty et al., *J. Chem. Soc.* 1067-68, 1994; Eichler et al., *Peptide Res.* 35 **7**:300-306, 1994). However, such cyclized peptides are

generally still quite flexible and are poorly bioavailable, and thus have met with only limited success.

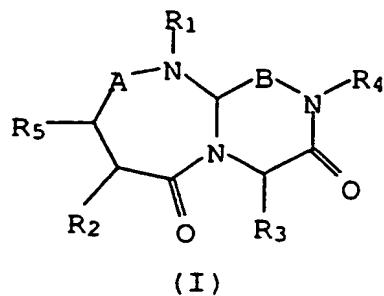
More recently, non-peptide compounds have been developed which more closely mimic the secondary structure 5 of reverse-turns found in biologically active proteins or peptides. For example, U.S. Patent No. 5,440,013 to Kahn and published PCT WO94/03494 to Kahn both disclose conformationally constrained, non-peptidic compounds which mimic the three-dimensional structure of reverse-turns.

10 While significant advances have been made in the synthesis and identification of conformationally constrained, reverse-turn mimetics, there is still a need in the art for small molecules which mimic the secondary structure of peptides. There is also a need in the art 15 for libraries containing such members, as well as techniques for synthesizing and screening the library members against targets of interest, particularly biological targets, to identify bioactive library members. The present invention fulfills these needs, and provides 20 further related advantages.

Summary of the Invention

In brief, the present invention is directed to conformationally constrained compounds which mimic the 25 secondary structure of reverse-turn regions of biologically active peptides and proteins. This invention also discloses libraries containing such compounds, as well as the synthesis and screening thereof.

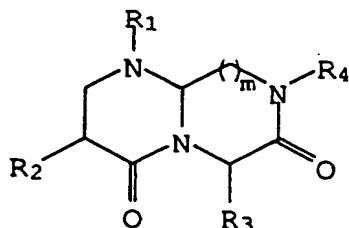
The compounds of the present invention have the 30 following general structure (I):



wherein A is $-(CHR')_n-$ and B is $-(CHR'')_m-$ where $n = 0, 1$ or
 5 2 and $m = 1, 2$ or 3 ; and wherein R' , R'' , R_1 , R_2 , R_3 , R_4 and
 R_5 are as defined in the following detailed description.

In one embodiment, $n = 0$, R'' and R_5 are hydrogen, and the compounds of this invention have the following structure (Ia):

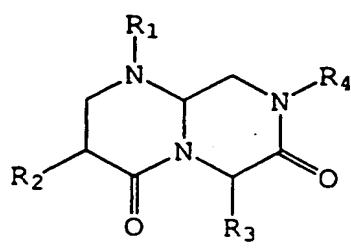
10



wherein $m = 1, 2$ or 3 and R_1 , R_2 , R_3 and R_4 are as defined
 15 in the following detailed description.

In a further embodiment, $n = 0$, R'' and R_5 are hydrogen, $m = 1$, and the compounds of the present invention have the following structure (Ib):

20



wherein R_1 , R_2 , R_3 and R_4 are as defined in the following detailed description.

The present invention is also directed to libraries containing compounds of structure (I) above, as well as methods for synthesizing such libraries and methods for screening the same to identify biologically active compounds.

These and other aspects of this invention will be apparent upon reference to the attached figures and following detailed description. To this end, various references are set forth herein which describe in more detail certain procedures, compounds and/or compositions, and are incorporated by reference in their entirety.

15 Brief Description of the Drawing

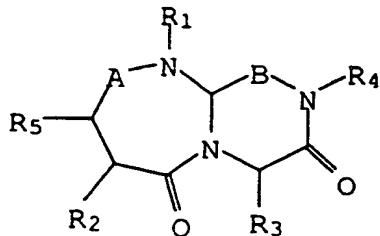
Figure 1 illustrates the percent inhibition of radioligand binding to δ and μ opiate receptors of a representative reverse-turn mimetic of this invention as a function of concentration.

20

Detailed Description of the Invention

The present invention is directed to reverse-turn mimetics and chemical libraries containing reverse-turn mimetics. The reverse-turn mimetics of the present invention are useful as bioactive agents, including (but not limited to) use as diagnostic, prophylactic and/or therapeutic agents. The reverse-turn mimetic libraries of this invention are useful in the identification of such bioactive agents. In the practice of the present invention, the libraries may contain from tens to hundreds to thousands (or greater) of individual reverse-turn mimetics (also referred to herein as "members").

In one aspect of the present invention, a reverse-turn mimetic is disclosed having the following structure (I):



(I)

where A is $-(CHR)_n-$, and B is $-(CHR')_m-$, n = 0, 1 or 2 and 5 m = 1, 2 or 3; and where R', R", R₁, R₂, R₃, R₄ and R₅ are as defined below.

In structure (I) above, the solid line designation for attachment of R₂, R₃, and R₅ indicates that these R groups may lie either above or below the plane of 10 the page. If a reverse-turn mimetic of this invention is intended to mimic a reverse-turn of naturally occurring amino acids (i.e., "L-amino acids"), the R groups would generally lie below the plane of the page (i.e., "—R") in Structure (I). However, if the reverse-turn mimetic of 15 this invention is intended to mimic a reverse-turn containing one or more D-amino acids, then the corresponding R group or groups would lie above the plane of the page (i.e., "—R") in Structure (I).

In one embodiment, R₁ and R₄ are the same or 20 different and represent the remainder of the compound, and R', R", R₂, R₃, and R₅ are the same or different and independently selected from an amino acid side chain moiety or derivative thereof. With regard to R' and R", it should be understood that each occurrence of R' and R" 25 is independently selected from amino acid side chain moieties or derivatives thereof. For example, when m=2, B is a $-CHR'CHR"-$ moiety. In this instance, both occurrences of R" are independently selected, and may be the same or different. Thus, if the first occurrence of 30 R" is hydrogen and the second methyl, B would have the structure $-CH_2CH(CH_3)-$.

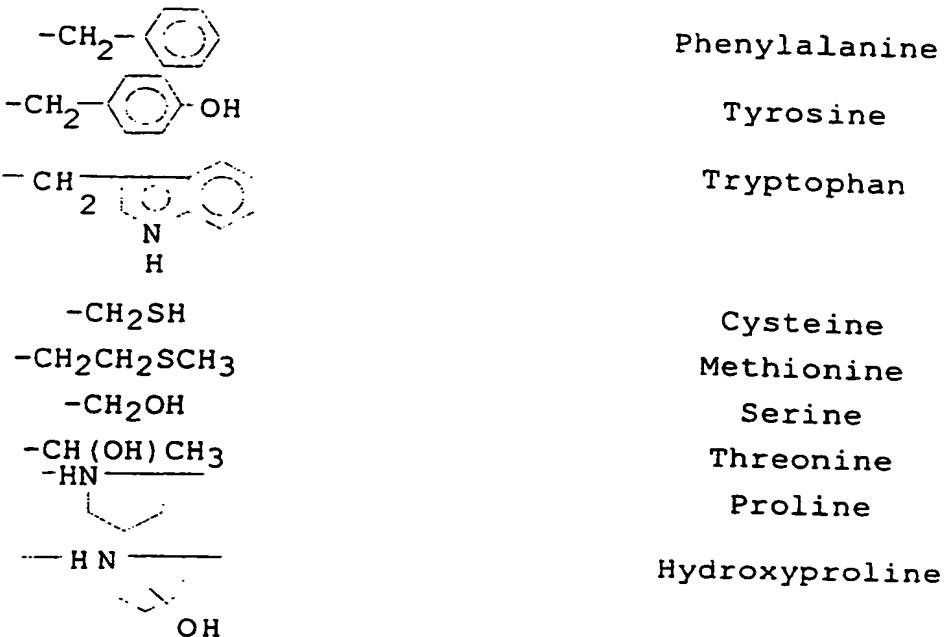
As used herein, the term "remainder of the compound" means any moiety, agent, compound, support, molecule, linker, amino acid, peptide or protein covalently attached to the reverse-turn mimetic at either 5 the R₁ and/or R₄ positions. This term also includes amino acid side chain moieties and derivatives thereof.

As used herein, the term "amino acid side chain moiety" represents any amino acid side chain moiety present in naturally occurring proteins including (but not 10 limited to) the naturally occurring amino acid side chain moieties identified in Table 1. Other naturally occurring amino acid side chain moieties of this invention include (but are not limited to) the side chain moieties of 3,5-dibromotyrosine, 3,5-diiodotyrosine, hydroxylysine, γ - 15 carboxyglutamate, phosphotyrosine and phosphoserine. In addition, glycosylated amino acid side chains may also be used in the practice of this invention, including (but not limited to) glycosylated threonine, serine and asparagine.

20

Table 1
Amino Acid Side Chain Moieties

<u>Amino Acid Side Chain Moiety</u>	<u>Amino Acid</u>
-H	Glycine
-CH ₃	Alanine
-CH(CH ₃) ₂	Valine
-CH ₂ CH(CH ₃) ₂	Leucine
-CH(CH ₃)CH ₂ CH ₃	Isoleucine
-(CH ₂) ₄ NH ₃ ⁺	Lysine
-(CH ₂) ₃ NHC(NH ₂)NH ₂ ⁺	Arginine
	Histidine
-CH ₂ COO ⁻	Aspartic acid
-CH ₂ CH ₂ COO ⁻	Glutamic acid
-CH ₂ CONH ₂	Asparagine
-CH ₂ CH ₂ CONH ₂	Glutamine



In addition to naturally occurring amino acid side chain moieties, the amino acid side chain moieties of the present invention also include various derivatives 5 thereof. As used herein, a "derivative" of an amino acid side chain moiety includes modifications and/or variations to naturally occurring amino acid side chain moieties. For example, the amino acid side chain moieties of 10 alanine, valine, leucine, isoleucine and phenylalanine may generally be classified as lower chain alkyl, aryl, or aralkyl moieties. Derivatives of amino acid side chain moieties include other straight chain or branched, cyclic or noncyclic, substituted or unsubstituted, saturated or unsaturated lower chain alkyl, aryl or aralkyl moieties.

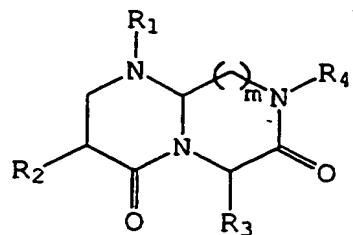
15 As used herein, "lower chain alkyl moieties" contain from 1-12 carbon atoms, "lower chain aryl moieties" contain from 6-12 carbon atoms and "lower chain aralkyl moieties" contain from 7-12 carbon atoms. Thus, in one embodiment, the amino acid side chain derivative is 20 selected from a C₁₋₁₂ alkyl, a C₆₋₁₂ aryl and a C₇₋₁₂ aralkyl, and in a more preferred embodiment, from a C₁₋₇ alkyl, a C₆₋₁₀ aryl and a C₇₋₁₁ aralkyl.

Amino side chain derivatives of this invention further include substituted derivatives of lower chain alkyl, aryl, and aralkyl moieties, wherein the substituent is selected from (but are not limited to) one or more of 5 the following chemical moieties: -OH, -OR, -COOH, -COOR, -CONH₂, -NH₂, -NHR, -NRR, -SH, -SR, -SO₂R, -SO₂H, -SOR and halogen (including F, Cl, Br and I), wherein each occurrence of R is independently selected from a lower 10 chain alkyl, aryl and aralkyl moieties. Moreover, cyclic lower chain alkyl, aryl and aralkyl moieties of this invention include naphthalene, as well as heterocyclic compounds such as thiophene, pyrrole, furan, imidazole, 15 oxazole, thiazole, pyrazole, 3-pyrroline, pyrrolidine, pyridine, pyrimidine, purine, quinoline, isoquinoline and carbazole. Amino acid side chain derivatives further 20 include heteroalkyl derivatives of the alkyl portion of the lower chain alkyl and aralkyl moieties, including (but not limited to) alkyl and aralkyl phosphonates and silanes.

In a further embodiment, and in addition to being an amino acid side chain moiety or derivative thereof, or the remainder of the compound in the case of R₁ and R₄, R₁, R₂, R₃, R₄, or R₅ may be a linker facilitating the linkage of the compound to another moiety or compound. 25 For example, the compounds of this invention may be linked to one or more known compounds, such as biotin, for use in diagnostic or screening assay. Furthermore, R₁, R₂, R₃, R₄ or R₅ may be a linker joining the compound to a solid support (such as a support used in solid phase peptide 30 synthesis) or alternatively, may be the support itself. In this embodiment, linkage to another moiety or compound, or to a solid support, is preferable at the R₁ or R₄ position, and more preferably at the R₄ position.

In a preferred embodiment, R₅ is hydrogen.

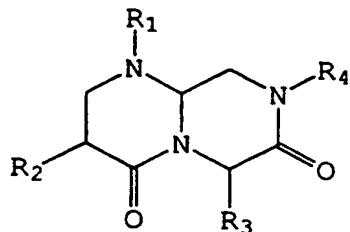
In one embodiment of this invention, $n=0$, R'' is hydrogen, and R_5 is hydrogen, and the reverse-turn mimetic has the following structure (Ia):



(Ia)

5 wherein $m = 1, 2$ or 3 and R_1, R_2, R_3 and R_4 are as defined above. In a preferred embodiment, $m = 1$ or 2 , R_1 and R_4 represent the remainder of the compound, and R_2 and R_3 are individually selected from an amino acid side chain moiety.

10 In another embodiment, $n=0$, $m=1$, R'' is hydrogen and R_5 is hydrogen, and the reverse-turn mimetic has the following structure (Ib):



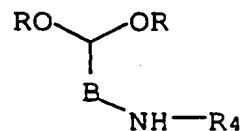
(Ib)

wherein R_1, R_2, R_3 and R_4 are as defined above. In a preferred embodiment, R_1 and R_4 represent the remainder of the compound, and R_2 and R_3 are independently selected from an amino acid side chain moiety.

15 The reverse-turn mimetics of the present invention may be prepared by utilizing appropriate 20 starting component molecules (hereinafter referred to as "component pieces"). Briefly, first and second component pieces are coupled to form a combined first-second

intermediate, third and fourth component pieces are coupled to form a combined third-fourth intermediate (or, if commercially available, a single third intermediate may be used), the combined first-second intermediate and 5 third-fourth intermediate (or third intermediate) are then coupled to provide a first-second-third-fourth intermediate (or first-second-third intermediate) which is cyclized to yield the reverse-turn mimetics of this invention. Alternatively, the reverse-turn mimetics may 10 be prepared by sequential coupling of the individual component pieces either stepwise in solution or by solid phase synthesis as commonly practiced in solid phase peptide synthesis.

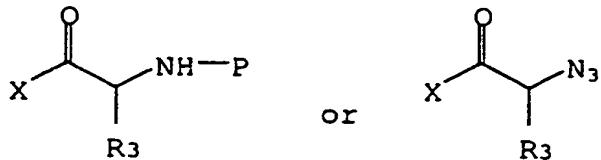
Within the context of the present invention, a 15 "first component piece" has the following structure 1:



1

where R_4 and B are as defined above, and R is a protective group suitable for use in peptide synthesis. Suitable R 20 groups include alkyl groups and, in a preferred embodiment, R is a methyl group. Such first component pieces may be readily synthesized by reductive amination by mating $\text{CH}(\text{OR})_2-(\text{CH}_2)_m-\text{CHO}$ with $\text{H}_2\text{N}-\text{R}_4$, or by displacement from $\text{CH}(\text{OR})_2-(\text{CH}_2)_m-\text{Br}$.

25 A "second component piece" of this invention has the following structure 2:

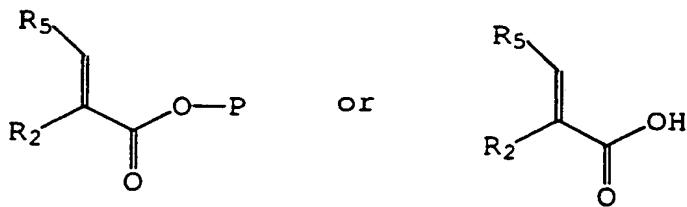


2

where R_3 is as defined above, P is an amino protective group suitable for use in peptide synthesis, and X represents the leaving group of the activated carboxylic acid group. Preferred protective groups include t-butyl dimethylsilyl (TBDMS), BOC, Fmoc, and Alloc (allyloxycarbonyl). N -Protected amino acids are commercially available. For example, Fmoc amino acids are available from a variety of sources. The conversion of these compounds to the second component pieces of this invention may be readily achieved by activation of the carboxylic acid group of the N -protected amino acid. Suitable activated carboxylic acid groups include acid halides where X is a halide such as chloride or bromide, acid anhydrides where X is an acyl group such as acetyl, reactive esters such as an N -hydroxysuccinimide esters and pentafluorophenyl esters, and other activated intermediates such as the active intermediate formed in a coupling reaction using a carbodiimide such as dicyclohexylcarbodiimide (DCC).

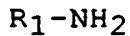
In the case of the azido derivative of an amino acid serving as the second component piece, such compounds may be prepared from the corresponding amino acid by the reaction disclosed by Zaloom et al. (J. Org. Chem. 46:5173-76, 1981).

A "third component piece" of this invention has the following structure 3:

3

where R_2 and R_5 are as defined above, and P is a carboxylic acid protective group such as a methyl or *t*-butyl group.

5 A "fourth component piece" of this invention has the following structure 4:



10

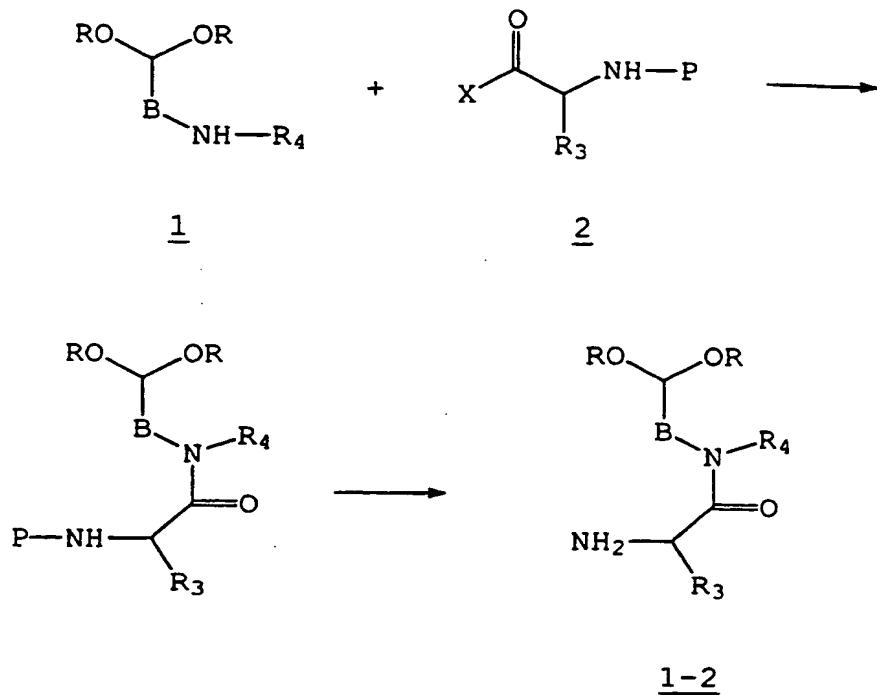
4

where R_1 is as defined above. Suitable fourth component pieces are commercially available from a variety of sources. Alternatively, the fourth component pieces may 15 be readily prepared by standard organic synthetic techniques commonly utilized for the synthesis of primary amines.

More specifically, the reverse-turn mimetics of this invention (see structure I above) are synthesized by 20 reacting a first component piece with a second component piece to yield a combined first-second intermediate, followed by either reacting the combined first-second intermediate with third and fourth component pieces sequentially, or reacting the intermediate with a combined 25 third-fourth intermediate to provide a combined first-second-third-fourth intermediate, and then cyclizing this intermediate to yield the reverse-turn mimetic.

The general synthesis of a reverse-turn mimetic having structure I may be synthesized by the following 30 technique. A first component piece 1 is coupled to a

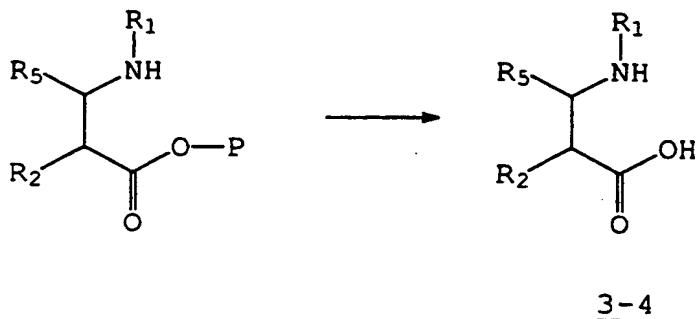
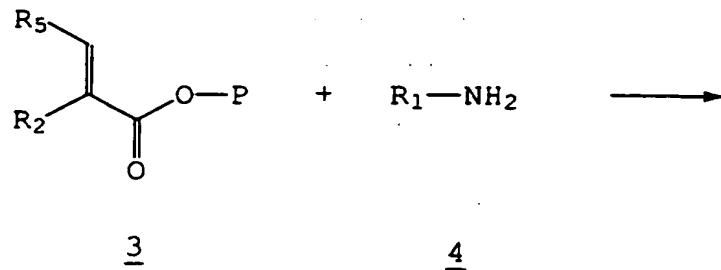
second component piece 2 to yield, after N-deprotection, a combined first-second intermediate 1-2 as illustrated below:



5

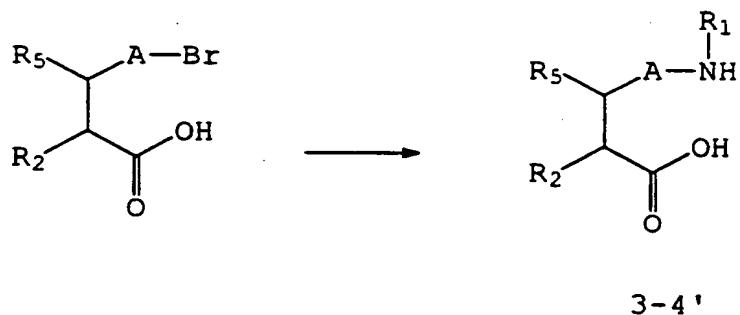
The synthesis of the reverse-turn mimetic may be convergent, in which case a combined third-fourth intermediate 3-4 is prepared from the coupling of a third component piece 3 with a fourth component piece 4 to yield, after O-deprotection, a combined third-fourth intermediate 3-4 as illustrated below:

15



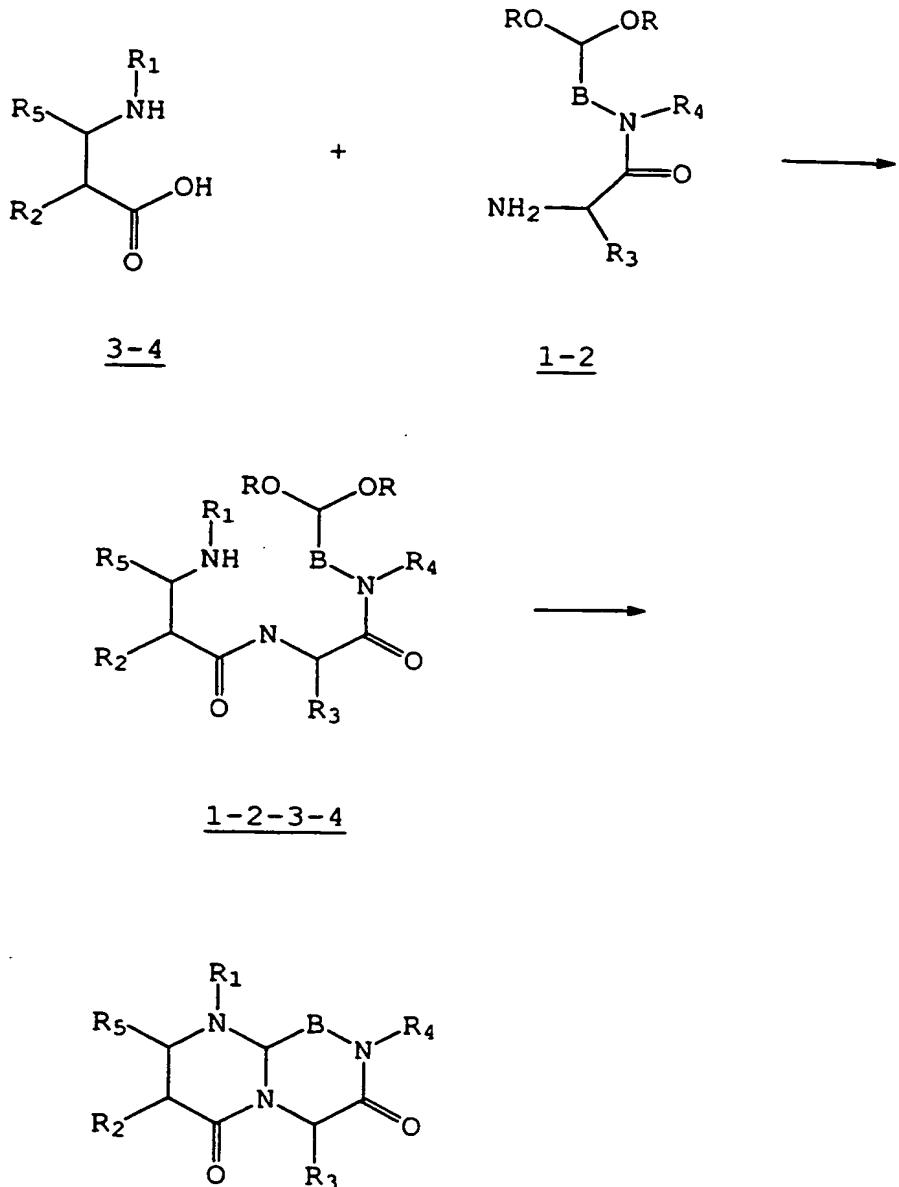
In the case where n of structure (I) above is 1 or 2, an intermediate of the following structure 3-4' can be made as follows:

5



wherein A is $-(\text{CH}_\text{R}')_n-$. Intermediate 3-4' may then be employed in place of intermediate 3-4 in the following reactions to yield a reverse-turn mimetic of this invention having structure (I).

Coupling of the combined intermediates 1-2 and 3-4 provides intermediate 1-2-3-4 which, upon cyclization, yield the reverse-turn mimetic (I) as illustrated below:



The syntheses of representative component pieces of this invention are described in Example 1. The

syntheses of representative combined first-second and third-fourth intermediates are described in Examples 2 and 3, respectively. The coupling of these intermediates to form a representative combined first-second-third-fourth 5 intermediate is described in Example 4. The cyclization of this intermediate to form a representative reverse-turn mimetic is described in Example 5.

As mentioned above, the reverse-turn mimetics of the present invention are useful as bioactive agents, such 10 as diagnostic, prophylactic, and therapeutic agents. The opiate receptor binding activity of a representative reverse-turn mimetic is presented in Example 7. In this example, the reverse-turn mimetic was found to effectively inhibit the binding of a radiolabeled enkephalin 15 derivative to the δ and μ opiate receptors. The data demonstrates the utility of these reverse-turn mimetics as receptor antagonists and as potential analgesic agents.

In another aspect of this invention, libraries containing reverse-turn mimetics of the present invention 20 are disclosed. Once assembled, the libraries of the present invention may be screened to identify individual members having bioactivity. Such screening of the libraries for bioactive members may involve, for example, evaluating the binding activity of the members of the 25 library or evaluating the effect the library members have on a functional assay. Screening is normally accomplished by contacting the library members (or a subset of library members) with a target of interest, such as, for example, an antibody, enzyme, receptor or cell line. Library 30 members which are capable of interacting with the target of interest are referred to herein as "bioactive library members" or "bioactive mimetics". For example, a bioactive mimetic may be a library member which is capable of binding to an antibody or receptor, which is capable of 35 inhibiting an enzyme, or which is capable of eliciting or antagonizing a functional response associated, for

example, with a cell line. In other words, the screening of the libraries of the present invention determines which library members are capable of interacting with one or more biological targets of interest. Furthermore, when 5 interaction does occur, the bioactive mimetic (or mimetics) may then be identified from the library members. The identification of a single (or limited number) of bioactive mimetic(s) from the library yields reverse-turn mimetics which are themselves biologically active, and 10 thus useful as diagnostic, prophylactic or therapeutic agents, and may further be used to significantly advance identification of lead compounds in these fields.

Synthesis of the peptide mimetics of the library of the present invention may be accomplished using known 15 peptide synthesis techniques, in combination with the first, second and third component pieces of this invention. More specifically, any amino acid sequence may be added to the N-terminal and/or C-terminal of the conformationally constrained reverse-turn mimetic. To this 20 end, the mimetics may be synthesized on a solid support (such as PAM resin) by known techniques (see, e.g., John M. Stewart and Janis D. Young, *Solid Phase Peptide Synthesis*, 1984, Pierce Chemical Comp., Rockford, Illinois) or on a silyl-linked resin by alcohol attachment 25 (see Randolph et al., *J. Am Chem. Soc.* 117:5712-14, 1995).

In addition, a combination of both solution and solid phase synthesis techniques may be utilized to synthesize the peptide mimetics of this invention. For example, a solid support may be utilized to synthesize the 30 linear peptide sequence up to the point that the conformationally constrained reverse-turn is added to the sequence. A suitable conformationally constrained reverse-turn mimetic which has been previously synthesized by solution synthesis techniques may then be added as the 35 next "amino acid" to the solid phase synthesis (i.e., the conformationally constrained reverse-turn mimetic, which

has both an N-terminus and a C-terminus, may be utilized as the next amino acid to be added to the linear peptide). Upon incorporation of the conformationally constrained reverse-turn mimetic into the sequence, additional amino 5 acids may then be added to complete the peptide bound to the solid support. Alternatively, the linear N-terminus and C-terminus protected peptide sequences may be synthesized on a solid support, removed from the support, and then coupled to the conformationally constrained 10 reverse-turn mimetic in solution using known solution coupling techniques.

In another aspect of this invention, methods for constructing the libraries are disclosed. Traditional combinatorial chemistry techniques (see, e.g., Gallop 15 et al., *J. Med. Chem.* 37:1233-1251, 1994) permit a vast number of compounds to be rapidly prepared by the sequential combination of reagents to a basic molecular scaffold. Combinatorial techniques have been used to construct peptide libraries derived from the naturally 20 occurring amino acids. For example, by taking 20 mixtures of 20 suitably protected and different amino acids and coupling each with one of the 20 amino acids, a library of 400 (i.e., 20^2) dipeptides is created. Repeating the procedure seven times results in the preparation of a 25 peptide library comprised of about 26 billion (i.e., 20^8) octapeptides.

In a further aspect of this invention, methods for screening the libraries for bioactivity and isolating bioactive library members are disclosed. The libraries of 30 the present invention may be screened for bioactivity by a variety of techniques and methods. Generally, the screening assay may be performed by (1) contacting a library with a biological target of interest, such as a receptor, and allowing binding to occur between the 35 mimetics of the library and the target, and (2) detecting the binding event by an appropriate assay, such as by the

colorimetric assay disclosed by Lam et al. (*Nature* 354:82-84, 1991) or Griminski et al. (*Biotechnology* 12:1008-1011, 1994) (both of which are incorporated herein by reference). In a preferred embodiment, the library 5 members are in solution and the target is immobilized on a solid phase. Alternatively, the library may be immobilized on a solid phase and may be probed by contacting it with the target in solution.

10 The following examples are provided for purposes of illustration, not limitation.

EXAMPLES

Example 1

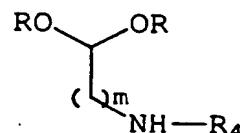
Synthesis of Component Pieces

In this example, the synthesis of representative component pieces which may be combined to form the reverse-turn mimetics of the present invention is 20 disclosed.

A. Representative First Component Pieces

A first component piece having the following structure 1 was utilized:

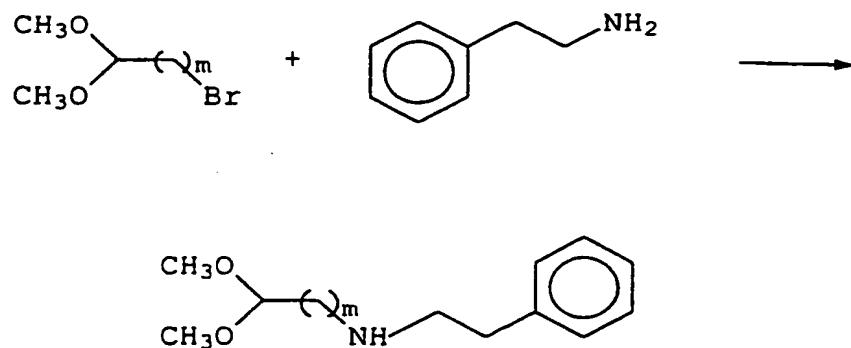
25



1

where R₄ is as defined above, and R represents a protective group suitable for use in peptide synthesis. Suitable R groups include alkyl groups and, in a preferred 30 embodiment, R is a methyl group.

Generally, the first component piece is prepared by N-alkylation of an amine with a dialkylacetal of a 2-haloethanal. The synthesis of a representative first component piece from phenethylamine and the dimethylacetal of 2-bromoethanal is depicted schematically below.

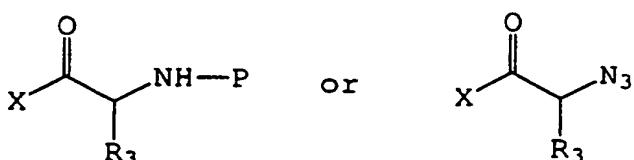


1a

In the procedure, 24 ml (3.43 ml, 20.3 mmol) of bromide and 2.8 ml (2.71 g. 22.3 mmol) phenethylamine was added 40 ml freshly distilled THF in a 150 ml argon charged round-bottom flask equipped with a reflux condenser. The reaction was heated at a gentle reflux for 24 hours, then volatiles were removed under reduced pressure and the residue was dissolved in 200 ml dichloromethane. The organic layer was washed with 2 x 100 ml sat. aq. sodium bicarbonate, sat. aq. sodium chloride, and dried over anhydrous sodium sulfate. Volatiles were removed under reduced pressure and the residue dried for 3 hrs. under high vacuum to yield 3.5 g (83%) first component piece la ($m=1$) as a light brown oil used without further purification.

B. Representative Second Component Pieces

A representative second component piece of this invention is a reactive N-protected amino acid having an activated carboxylic acid group, or an azido derivative of 5 an amino acid, as represented by the following structure 2:



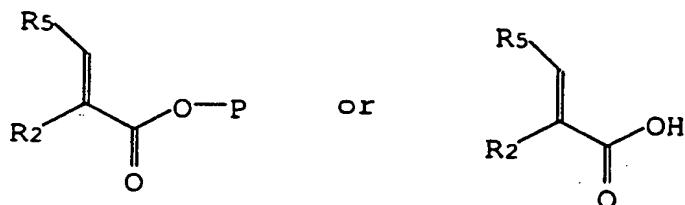
2

where R₃ is as defined above, P is an amino protective 10 group suitable for use in peptide synthesis, and X represents the leaving group of the activated carboxylic acid group. Preferred protective groups include t-butyl dimethylsilyl (TBDMS), BOC, Fmoc, and Alloc (allyloxycarbonyl). N-Protected amino acids are 15 commercially available. For example, Fmoc amino acids are available from a variety of sources. The conversion of these compounds to the second component pieces of this invention may be readily achieved by activation of the carboxylic acid group of the N-protected amino acid. 20 Suitable activated carboxylic acid groups include acid halides where X is a halide such as chloride or bromide, acid anhydrides where X is an acyl group such as acetyl, reactive esters such as an N-hydroxysuccinimide esters and p-nitrophenyl esters, and other activated intermediates 25 such as the active intermediate formed in a coupling reaction using a carbodiimide such as dicyclohexylcarbodiimide (DCC). Similarly, the corresponding azido derivative may be prepared by known techniques. In a preferred embodiment, X is hydroxyl for 30 HATU (N-(7-azabenzotriaol-1-yl)-1,1,3,3-tetramethyluronium

hexafluorophosphate) coupling, or is fluorine for silicon mediated coupling.

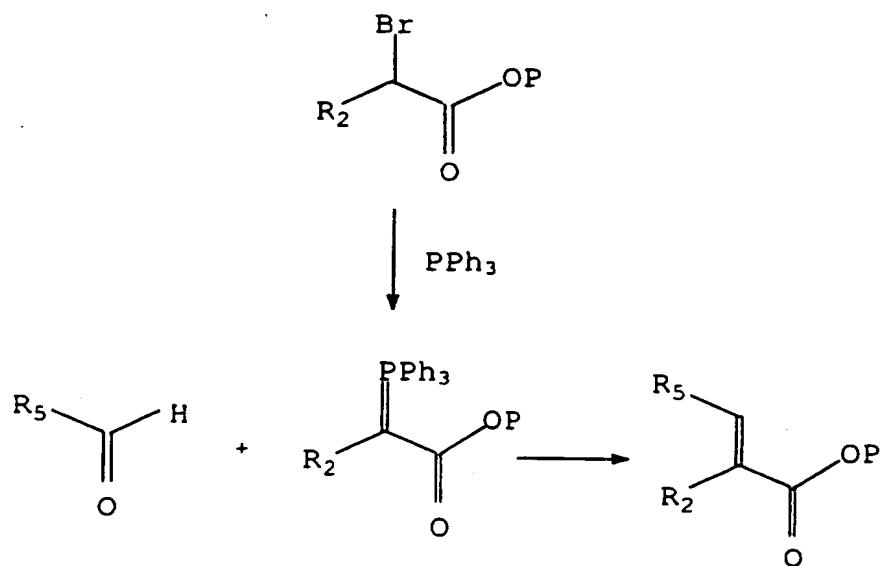
C. Representative Third Component Pieces

5 A representative third component piece of this invention is an α,β -unsaturated carboxylic acid or derivative thereof having the following structure 3:



3

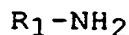
10 where R₂ and R₅ are as defined above, and P is a carboxylic acid protective group such as a methyl or t-butyl group. Such third component pieces may be obtained commercially, or synthesized from the commercially available aldehyde and the appropriate phosphorusylide according to the 15 following reaction scheme:



(see, Wadsworth and Emmons, *Org. Syn.* 45:44, 1965).

D. Representative Fourth Component Pieces

5 A representative fourth component piece of this invention is a primary amine having the following structure 4:



10

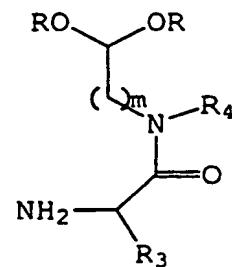
4

15 where R_1 is as defined above. Suitable fourth component pieces are commercially available from a variety of sources. Alternatively, the fourth component pieces may be readily prepared by standard organic synthetic techniques commonly utilized for the synthesis of primary amines.

Example 220 Combined First-Second Intermediates: The Coupling of
First and Second Component Pieces

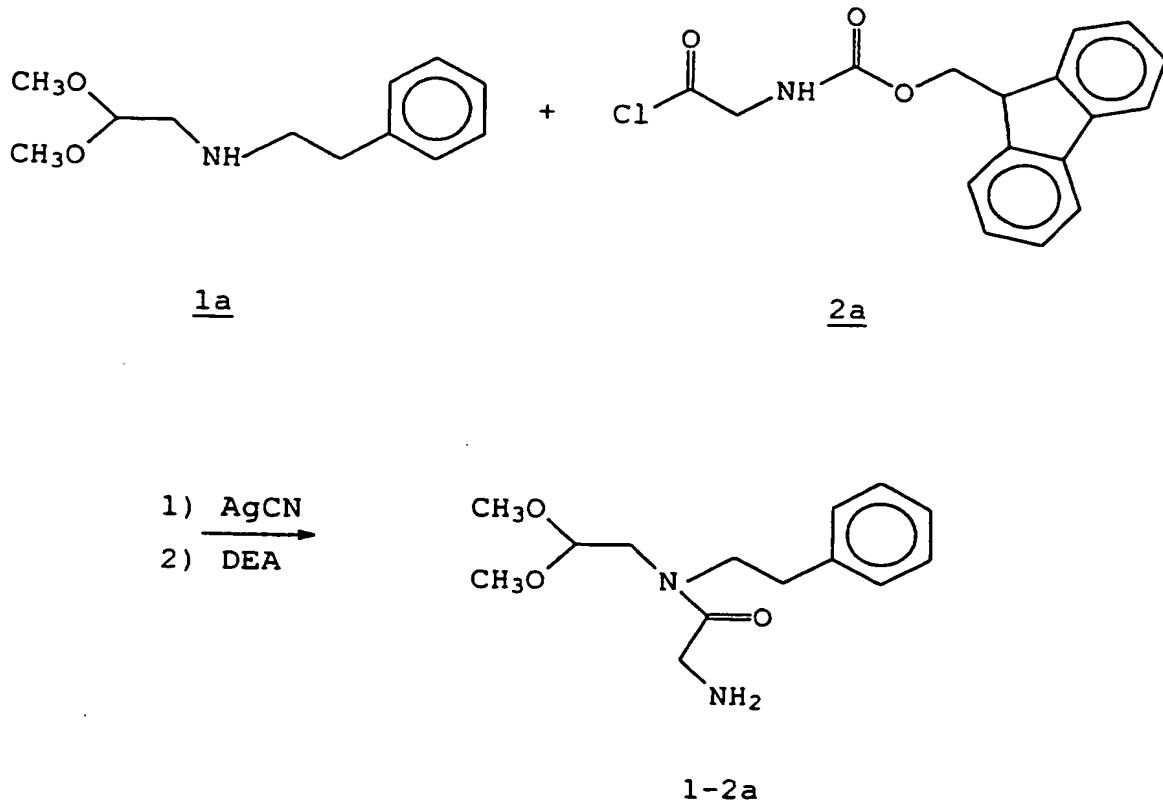
25 The coupling of the component pieces to produce the reverse-turn mimetics of the present invention generally involve the formation of amide bonds. The amide bonds which link the pieces may be formed by standard synthetic peptide techniques and may be performed by either liquid or solid phase synthesis.

30 The coupling of the first and second component pieces provides, after deprotection, a combined first-second intermediate having the following structure 1-2:

1-2

where R, R₃, and R₄ are as described above (in this example, R" of structure (I) is/are hydrogen).

The preparation of a combined first-second intermediate is accomplished by amide bond formation between the amine of a first component piece 1 and the activated carboxylic acid group of a second component piece 2 followed by N-deprotection. The synthesis of a representative combined first-second intermediate is 10 depicted schematically below.



In the procedure, to 650 mg (3.17 mmol) first component piece 1a prepared as described in Example 1A and 1 g (3.17 mmol) FMOC-glycine chloride, 2a, 10 ml freshly distilled benzene in a 25 ml argon charged round bottom flask was added 937 mg (7 mmol) silver cyanide (AgCN), and the resulting reaction mixture was stirred vigorously for 48 hrs. The reaction was diluted to 25 ml w/ethyl acetate and filtered through a Celite plug. Volatiles were removed under reduced pressure and the residue was chromatographed using 20:80 ethyl acetate:hexane as the mobile phase over flash grade silica gel to yield 1.1 g (71%) of an amorphous solid.

To 400 mg (0.82 mmol) of the amorphous solid in 5 ml acetonitrile was added 1 ml diethylamine (DEA) dropwise and the resulting reaction mixture was stirred at room

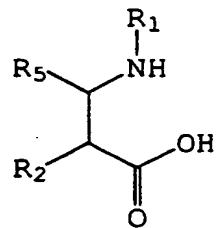
temperature for 2 hrs. The volatiles were removed under reduced pressure and the residue was chromatographed using 5% methanol saturated with ammonia 95% dichloromethane as the mobile phase over flash grade silica gel to yield 207 5 mg (95%) of a combined first-second intermediate, 1-2a, as a thick colorless oil.

Example 3

10 Combined Third-Fourth Intermediates: The Coupling of Third and Fourth Component Pieces

The coupling of a third component piece with a fourth component piece provides a combined third-fourth intermediate. The combined third-fourth component piece 15 is produced by amine bond formation resulting from the conjugate addition of the amine group of a fourth component piece 4 to the α,β -unsaturated carbonyl group of a third component piece 3.

The coupling of third and fourth component pieces 20 provides, after deprotection, a combined third-fourth intermediate having the following structure 3-4:

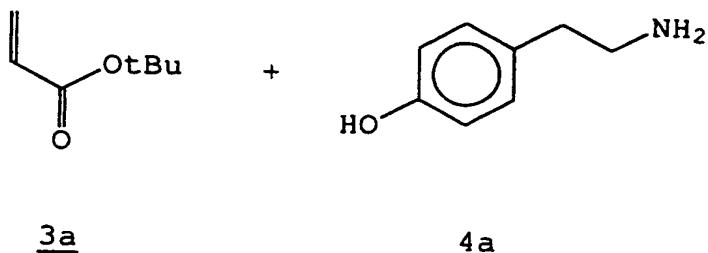


3-4

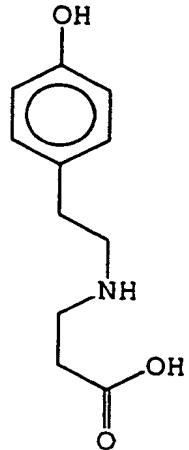
where R₁, R₂, and R₅ are as described above (in this 25 example, n of structure (I) is 0).

The preparation of a combined third-fourth intermediate is accomplished by amine bond formation between the primary amino group of a fourth component piece 4 and α,β -unsaturated carbonyl group of a third

component piece 3 followed by O-deprotection. The synthesis of a representative combined third-fourth intermediate is depicted schematically below.



1) Methanol/THF
 2) TFA
 3) neutral alumina



3-4a

5

In the procedure, to 5 g of tyramine suspended in 40 ml freshly distilled tetrahydrofuran (THF) in an argon charged, 250 ml round-bottom flask was added methanol sufficient to dissolve the suspension. To the resulting 10 solution was added 5.3 ml (4.67 g, 36.4 mmol) of t-butylacrylate dropwise over the course of 5 min, and the resulting reaction mixture was stirred overnight at room 15 temperature. An additional 2 ml of t-butylactylate was added to consume the remaining starting material and the reaction was stirred an additional 4 hrs. Volatiles were removed under reduced pressure and the residue was

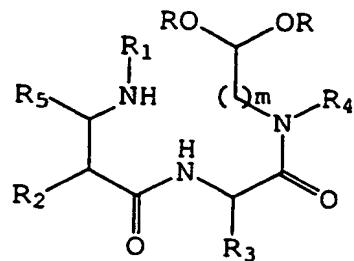
chromatographed using 95:5 dichloromethane:ammonia saturated methanol:NH₃/MeOH as the mobile phase over flash grade silica gel to yield 6.6 g (68%) of the ester, a colorless oil which solidified upon overnight refrigeration. To a solution of 1 gram (3.77 mmol) of the ester in 20 ml dichloromethane at 0°C was added 80 ml of cold trifluoroacetic acid (TFA) and the resulting reaction mixture was stirred with warming to room temperature over the course of 24 hrs. Volatiles were removed under reduced pressure to yield 950 mg of a clear oil. The end product was dissolved in 95:5 dichloromethane:methanol and slowly filtered through a pad of neutral alumina. Volatiles were removed from the filtrate to yield 750 mg of 3-4a as an amorphous solid.

15

Example 4Combined First-Second-Third-Fourth Intermediates: The Coupling of Combined First-Second and Third-Fourth Intermediates

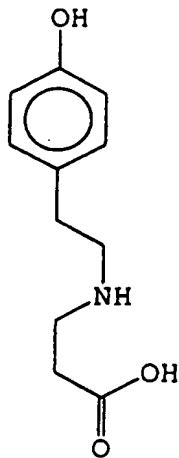
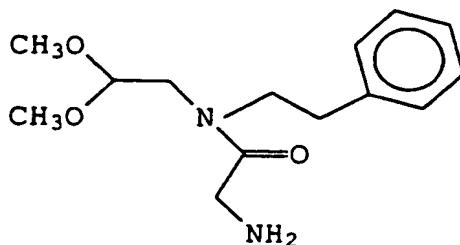
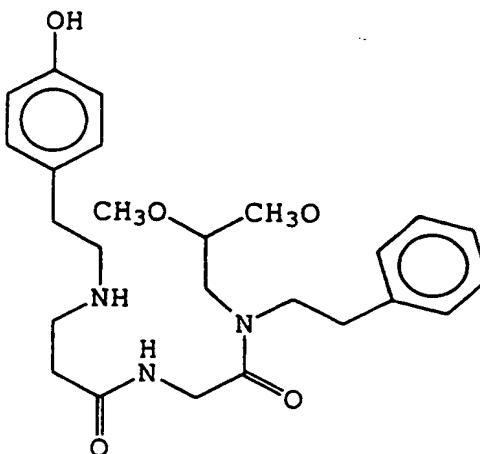
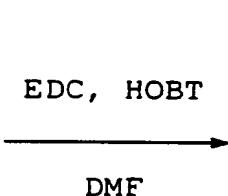
20

The coupling of a combined first-second intermediate with a combined third-fourth intermediate provides a combined first-second-third-fourth intermediate. The combined first-second-third-fourth intermediate is produced by amide bond formation resulting from the coupling of the amine group of a combined first-second intermediate 1-2 to the carboxylic acid group of a combined third-fourth intermediate 3-4. The combined first-second-third-fourth intermediate has the following structure 1-2-3-4:

1-2-3-4

where R , R_1 , R_2 , R_3 , R_4 and R_5 are as described above.

The synthesis of a representative combined first-second-third-fourth intermediate is depicted schematically 5 below.

3-4a1-2a1-2-3-4a

In the procedure, 212 mg (1.0 mmol) 3-4a, 270 mg (1.01 mmol) 1-2a, and 136 mg (1.01 mmol) 1-hydroxybenzotriazole hydrate (HOBT) were dissolved in 10 ml dimethylformamide (DMF) and cooled to 0°C. To this solution was added 290 mg (1.52 mmol, 1.5 eq) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and the resulting reaction mixture was stirred and warmed to room temperature over the course of 24 hours. The DMF was

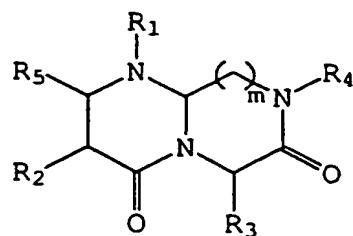
removed under reduced pressure and the residue was redissolved in 200 ml ethyl acetate. The ethyl acetate layer was washed with saturated aqueous sodium bicarbonate, water, and dried over anhydrous sodium sulfate. Volatiles were removed under reduced pressure and the residue was chromatographed using 95:5 dichloromethane: ammonia saturated methanol as eluent over flash-grade silica gel to yield 310 mg (0.68 mm 67%) 1-2-3-4a as a thick colorless oil.

10

Example 5The Synthesis of a Representative Reverse-Turn Mimetic:
Cyclization of a Combined First-Second-Third-Fourth
Intermediate

15

The cyclization of a combined first-second-third-fourth intermediate provides a reverse-turn mimetic of the present invention. The combined first-second-third-fourth intermediate 1-2-3-4 is cyclized by treatment with camphorsulfonic acid (CSA) or, in a preferred embodiment, TMSOTF (at 0°C) to provide a reverse-turn mimetic having the following structure (Ia):



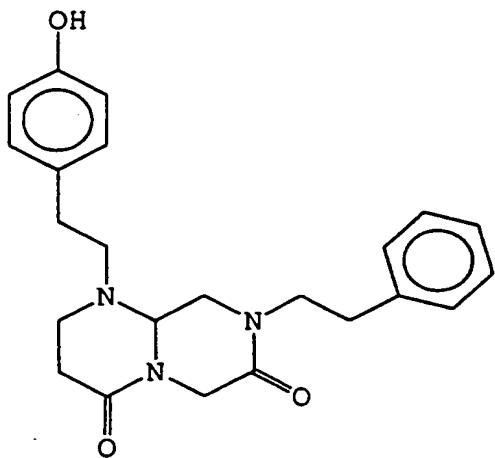
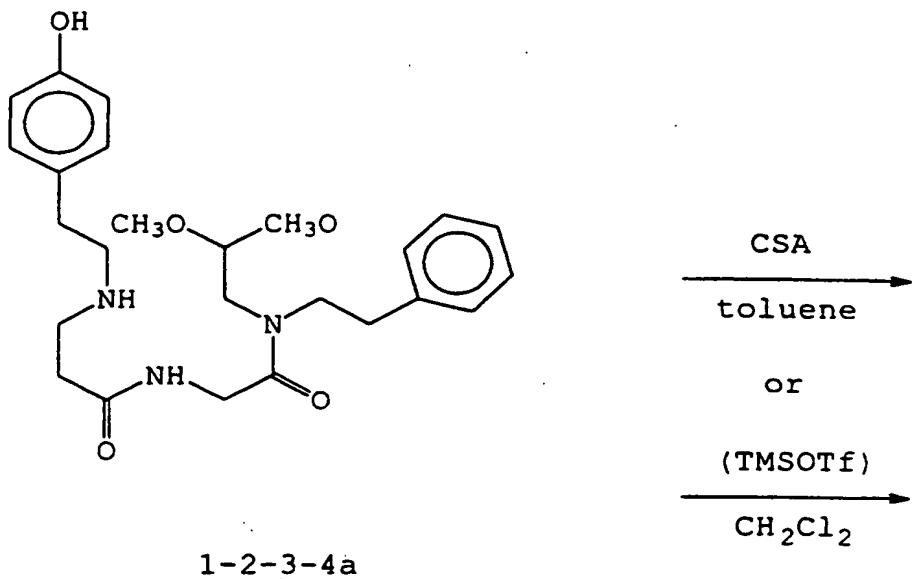
(Ia)

25

where R₁, R₂, R₃, R₄, and R₅ are as described above.

The synthesis of a representative reverse-turn mimetic of the present invention is depicted schematically below.

30

Ia

In the procedure, 0.5 g (2.4 mmol) camphorsulfonic acid (CSA) was azeotroped with 3-15 ml portions of freshly 5 distilled toluene and dried under vacuum at 40°C for 3 hrs in a 100 ml round-bottom flask equipped with a reflux condenser. Then 20 ml of freshly distilled toluene was added and the CSA solution was heated to a vigorous reflux. To this refluxing CSA solution was added a

solution of 50 mg (0.11 mmol) 1-2-3-4a in 20 ml of freshly distilled toluene by syringe pump over the course of 1 hr. The resulting reaction mixture was refluxed for 12 hrs, cooled to room temperature and diluted to 200 ml
5 ethylacetate. The organic layer was washed with 2-75 ml portions of saturated aqueous sodium bicarbonate, 75 ml saturated aqueous sodium chloride, and dried over anhydrous sodium sulfate. Volatiles were removed under reduced pressure to yield 22 mg of Ia as a glassine solid.
10 The crude product was triturated with 50/50 diisopropyl ether:hexane to remove non-polar impurities. The solid was then dissolved in dichloromethane and filtered to remove polar impurities. The residue upon evaporation was dried *in vacuo* for 24 hrs.

15

Example 6
The Synthesis of a Representative
Reverse-Turn Mimetic Salt

20 The reverse-turn mimetics of the present invention are nitrogen bases and may, therefore, be converted to their corresponding salts by treatment with various acids. In this example, the preparation of a representative salt of a reverse-turn mimetic is described.

25 The 2,4-dinitrobenzoic acid salt of reverse-turn mimetic Ia, prepared as described in Example 5, was obtained by treatment of the reverse-turn mimetic with the acid in aqueous methanol. In the procedure, 5 mg (12.7 μ mol) Ia was dissolved in 3 ml of 80/20 methanol:water and
30 cooled to 0°C. To this solution was added 2.70 mg (12.7 μ mol, 1.0 eq) 2,4 dinitrobenzoic acid, and the resulting solution stirred until it became homogenous. Volatiles were removed under reduced pressure and the residue was dried *in vacuo* for 24 hrs. The residue was taken up in
35 warm water and filtered to remove insoluble impurities. The salt was then lyophilized.

Example 75 Activity of a Representative Reverse-Turn
Mimetic in Opioid Receptor Binding

In this example, the binding activity of a representative reverse-turn mimetic to the delta (δ) and mu (μ) opioid receptors is described. In these methods, the 10 binding of the 2,4-dinitrobenzoic acid salt of reverse-turn mimetic of structure Ia, prepared as described in Example 6, was evaluated in competitive radioligand binding assays.

15 A. Opiate (δ) Binding Activity

In this method, membranes were prepared from whole brains of male guinea pigs and equilibrated with 2 nM [3 H]DPDPE (D-pen³, D-pen⁵) enkephalin for 1 hour at 4°C after which test substances were added and incubated for 4 20 hours at 25°C. Non-specific binding was determined in the presence of 0.3 μ M naltrindole. Bound [3 H]DPDPE was separated from free radioligand by rapid filtration through glass fiber filtermats and subsequently washed 3 times. Filtermats were then counted in the LKB Betaplate 25 to determine specifically bound [3 H]DPDPE. (See Mosberg et al., "Structural Requirements for δ Opiate Receptor Binding," *Molec. Pharmacol.* 31:599-602, 1987.)

Table 230 Effect of Reference Compounds on [3 H]DPDPE Bound (2nM)

Compound	IC ₅₀ (nM)	Ki (nM)	Hill Coefficient
DAMGO	4,800	1,200	1.08
DPDPE	5.5	1.3	0.86
Naltrindole	0.63	0.20	0.53
U-50488	53,000	16,000	0.73

In this assay, the radioligand, [³H]DPDPE, was determined to have a $K_d = 0.65$ nM with a $B_{max} = 12.6$ fmol/mg protein and a specific binding of 60%. At a concentration of 10 μ M, the 2,4-dinitrobenzoic acid salt of reverse-turn mimetic Ia was found to inhibit radioligand binding at the 60% level, and exhibited a $K_i = 1.7 \pm 0.3$ μ M and an $IC_{50} = 6.9 \pm 1.2$ μ M. These results are presented in Figure 1 (o) which depicts the % inhibition of radioligand binding as a function of reverse-turn mimetic Ia concentration. These results demonstrate that reverse-turn mimetic Ia, in particular, and the reverse-turn mimetics of the present invention, in general, effectively inhibit binding to the δ opiate receptor, and possesses analgesic activity.

15

B. Opiate (μ) Binding Activity

In this method, membranes were prepared from whole brains of male guinea pigs and incubated with 2 nM [³H]DAMGO (D-Ala², N-methyl-phe⁴, gly-ol⁵)-enkephalin) for 2 hours at 25°C. Non-specific binding was determined in the presence of 0.5 μ M DAMGO. Bound [³H]DAMGO was separated from free radioligand by rapid filtration through glass fiber filtermats and subsequently washed 3 times. Filtermats were then counted in the LKB Betaplate to determine specifically bound [³H]DAMGO. (See Patricia et al., "Pharmacological profiles of fentanyl analogs at μ , δ and κ opiate receptors," *Eur. J. Pharmacol.* **213**:219-225, 1992.)

Table 3
Effect of Reference Compounds on [³H]DAMGO Bound (2nM)

Compound	IC ₅₀ (nM)	K _i (nM)	Hill Coefficient
DAMGO	6.5	0.59	0.92
DPDPE	4.0	0.37	1.32
Fentanyl	14	1.2	0.99
Naloxone	9.3	0.76	1.09
Naltrindole	27	2.5	0.98
Norbinaltorphimine	280	26	1.13
U-50488	6.1	0.59	0.70

5 In this assay, the radioligand, [³H]DAMGO, was determined to have a K_d = 0.27 nM with a B_{max} = 8.7 pmol/mg protein and a specific binding of 70%. At a concentration of 10 μ M, the 2,4-dinitrobenzoic acid salt of reverse-turn mimetic Ia inhibited radioligand binding at the 64% level, 10 and exhibited a K_i = 0.64 \pm 0.08 μ M and an IC₅₀ = 5.4 \pm 0.7 μ M. These results are presented in Figure 1 (•) which depicts the % inhibition of radioligand binding as a function of reverse-turn mimetic Ia concentration. These 15 results demonstrate that reverse-turn mimetic Ia, in particular, and the reverse-turn mimetics of the present invention, in general, effectively inhibit binding to the μ opiate receptor, and possesses analgesic activity.

Example 8

20 In Vitro Activity of a Representative
Reverse-Turn Mimetic for Analgesic Activity

 In this example, the *in vivo* activity of a representative reverse-turn mimetic as an analgesic agent 25 is presented. The 2,4-dinitrobenzoic acid salt of the reverse-turn mimetic of structure Ia, prepared as described in Example 6 (hereinafter referred to as "test

compound"), was utilized in the mouse tail flick assay (PanLabs, Pharmascreen Test No. 10402A). In this assay, the time required to elicit a tail-flick response to radiant heat pain stimulus in a group of mice is measured 5 as the pain threshold response.

Groups of five (3 test groups + 1 saline control + 1 morphine positive control) male ICR mice weighing 22 (± 2) grams each were used. Each of these animals were pre-selected and elicited a tail flick response within 6-10 7.5 seconds after a focused beam of radiant heat was focused on the middle dorsal surface of the animal's tail. Specific amounts of the test compound (i.e., 10, 30 and 100 μ g) were dissolved in 5 microliters (5 μ l) saline containing 6% DMSA and administered 15 intracerebroventricularly (ICV) to each animal. A saline-only solution was used as a negative control, with an ICV injection of 10 μ g/5 μ l/animal of morphine serving as a positive control.

At one minute post-ICV injection, the groups of mice 20 were measured for tail flick response, with a maximum cut-off time of 15 seconds. The mean of the response time for each treatment groups was calculated for a comparison between pre-treatment ("0 time") and 1 minute post-treatment 1 ("1 min."). Prolongation 1 minute post-25 treatment of over 50% ("% Prolong.") was considered significant activity. The results of this experiment are presented in Table 4, and demonstrate that the test compound had significant analgesic activity (i.e., approximately 10%-15% the potency of morphine).

30

Table 4
In Vivo Tail Flick Assay

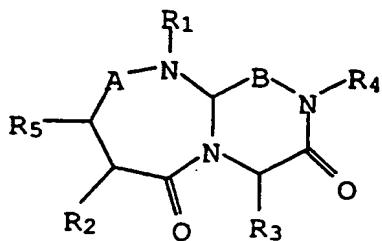
Compound	Dose/5 μ l	0 Time	1 Min.	% Prolong.
Saline	0	6.9	6.7	--
		6.9	7.5	--

		6.1	6.2	--
		6.5	6.3	--
		Avg.=6.6	Avg.=6.7	2%
Morphine	10 μ g	7.5	>15	--
		6.3	>15	--
		7.2	>15	--
		6.8	>15	--
		Avg.=7.0	Avg.>15	100%
Test Compound	100 μ g	6.5	>15	--
		6.3	>15	--
		6.5	>15	--
		6.8	>15	--
		Avg.=6.5	Avg.>15	100%
	30 μ g	6.5	>15	--
		6.7	7.2	--
		7.2	6.3	--
		6.3	>15	--
		Avg.=6.7	Avg.>15	63%
	10 μ g	6.5	7.5	--
		7.2	7.5	--
		6.9	6.7	--
		6.2	6.8	--
		Avg.=6.7	Avg.7.1	6%

It will be appreciated that, although specific embodiments of the invention have been described herein for the purposes of illustration, various modifications 5 may be made without departing from the spirit and scope of the invention. Accordingly, the invention is not limited except by the appended claims.

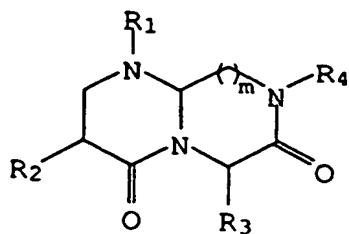
Claims

1. A compound having the structure:



wherein A is $-(CHR')_n-$, B is $-(CHR'')_m-$, n=0, 1 or 2 and m=1, 2 or 3; R', R'', R₂, R₃ and R₅ are the same or different and independently selected from an amino acid side chain moiety or derivative thereof, a linker and a solid support; and R₁ and R₄ represent the remainder of the compound.

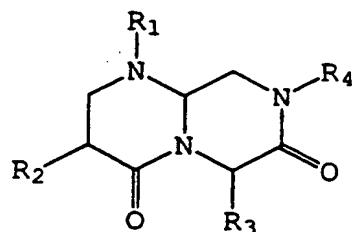
2. The compound of claim 1 wherein R' is hydrogen.
3. The compound of claim 1 wherein R'' is hydrogen.
4. The compound of claim 1 wherein n=0.
5. The compound of claim 1 wherein m=1 or 2.
6. The compound of claim 1 wherein R₅ is hydrogen.
7. The compound of claim 1 wherein n=0, R'' and R₅ are hydrogen, and the compound has the structure:



8. The compound of claim 7 wherein R₂ and R₃ are the same or different and independently selected from an amino acid moiety or derivative thereof.

9. The compound of claim 7 wherein R₄ is joined to a solid support.

10. The compound of claim 1 wherein n=0, R" and R₅ are hydrogen, m=1, and the compound has the structure:



11. The compound of claim 10 wherein R₂ and R₃ are the same or different and independently selected from an amino acid moiety or derivative thereof.

12. The compound of claim 10 wherein R₄ is joined to a solid support.

13. A library of compounds, comprising at least one compound of any one of claims 1 through 12.

14. A method of identifying a biologically active compound, comprising screening the library of claim 13 to identify the biologically active compound.

1/1

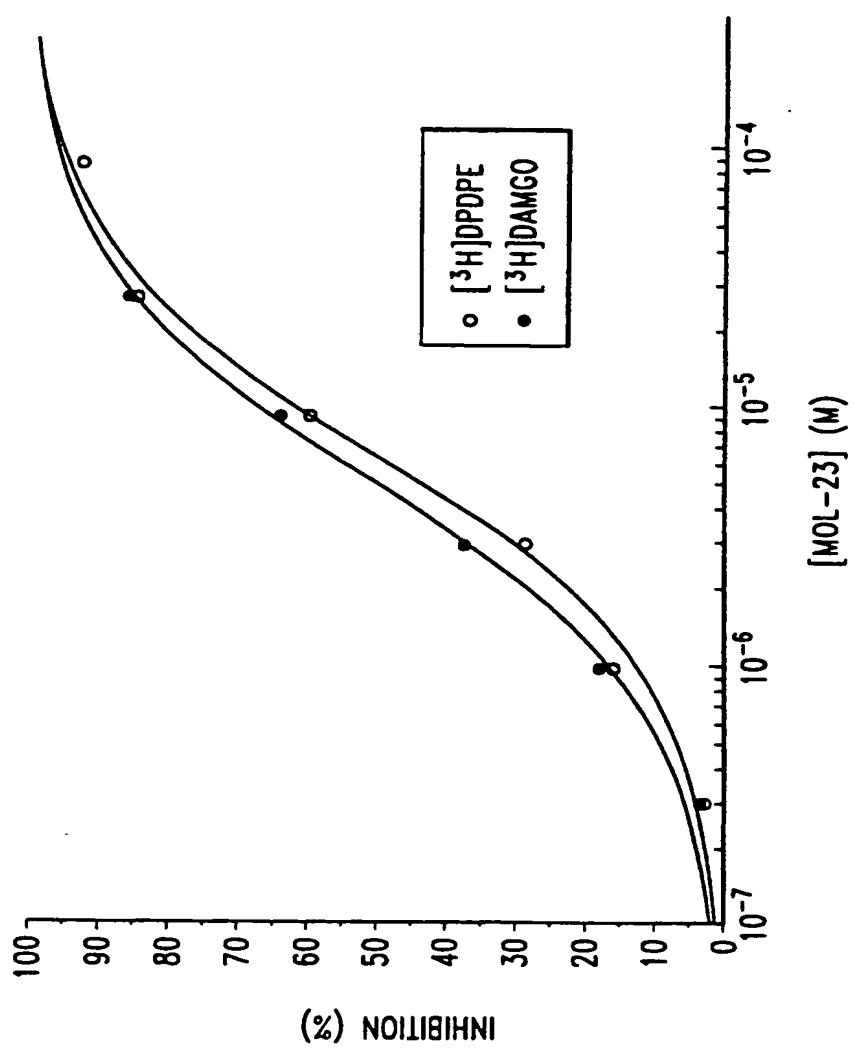


Fig. 1

INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/US 96/17053

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D487/04 A61K31/495 // (C07D487/04, 241:00, 239:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 440 013 A (KAHN MICHAEL) 8 August 1995 cited in the application see the whole document ---	1-14
A	WO 94 03494 A (UNIV PENNSYLVANIA) 17 February 1994 cited in the application see the whole document ---	1-14
A	JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 115, no. 19, 1993, DC US, pages 8861-62, XP002025257 T.W. KU ET AL.: "Direct design of a potent non-peptide fibrinogen receptor antagonist" -----	1-14

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

1

Date of the actual completion of the international search

14 February 1997

Date of mailing of the international search report

24.02.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

De Jong, B

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int'l	Application No
	PCT/US 96/17053

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-5440013	08-08-95	AU-A-	1570292	07-09-92
		AU-A-	3066495	18-01-96
		CA-A-	2103577	08-08-92
		EP-A-	0573608	15-12-93
		JP-T-	6505486	23-06-94
		WO-A-	9213878	20-08-92
		US-A-	5475085	12-12-95
<hr style="border-top: 1px dashed black;"/>				
WO-A-9403494	17-02-94	AU-A-	5000693	03-03-94
		CA-A-	2141447	17-02-94
		EP-A-	0656907	14-06-95
		JP-T-	7509723	26-10-95
		US-A-	5475085	12-12-95
<hr style="border-top: 1px dashed black;"/>				